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REVIEW

Nutrient responses as a key factor to the ecology of orchid species

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Key-words: nutrient metabolism, nutrient responses, orchid life cycles, orchid mycorrhiza.

INTRODUCTION

The availability of N and P plays a critical role in orchid growth and survival; the aim of the present review is to give an overview over the mechanisms which contribute to the critical response.

In the first heterotrophic and subterranean phase of orchid development, growth is entirely dependent on mycorrhizal fungi (for reviews see Hadley 1982; Harley & Smith 1983; Hadley & Pegge 1989). The nutrient metabolism of developing orchid individuals is adapted to this symbiosis: reductions in orchid nitrogen metabolism are permitted which can be considered adaptations to the parasitic habit during at least this phase (cf. Press *et al.* 1986). Furthermore, also in later life the nitrogen and phosphate fluxes are thoroughly affected (Alexander & Hadley 1984; Alexander *et al.* 1984). As a consequence, nutrient effects on orchid growth may occur via the symbiotic association, and may originate by affecting growth of the symbiotic fungi, or by affecting the symbiotic interaction between orchid and fungi (Dijk & Eck 1995c). Changes in orchid nutrient metabolism during the course of development reflect changes in the physiology of the symbiosis. The resulting development in orchid nutrient metabolism forms a further need to distinguish between the various stages in the life cycle, most notably between the germination and seedling stage and the C-autotrophic adult stage‡. Once above ground, orchids as slowly growing and often low, rosette-forming species are very sensitive to eutrophication, which favours more competitive species to a much larger degree than orchids (Dijk & Olff 1994; Silvertown *et al.* 1994).

‡Stages of development are defined as follows: germination: swelling of embryo due to uptake of water, rupture of seed coats and rhizoid formation on a not much enlarged embryo; protocorm: heterotrophic stage, lacking a genuine tuber, shoot and leaves; and seedlings: individuals having formed a shoot, later also a first, dormant genuine tuber. After breaking of dormancy of the first genuine tuber, individuals are named adults.

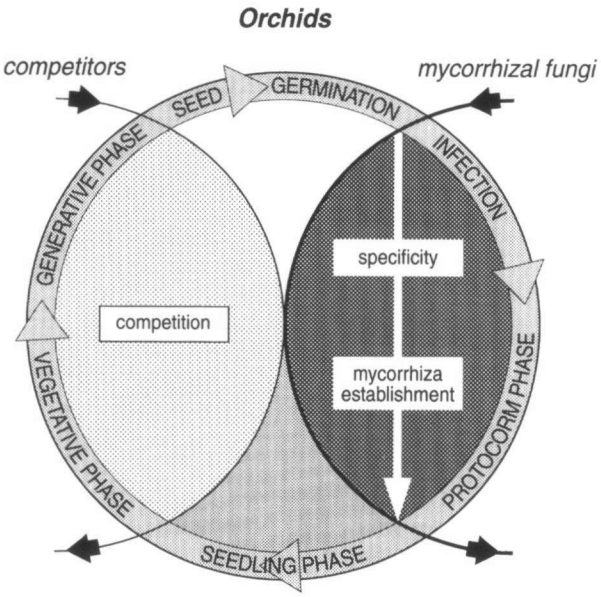

Fig. 1. The various stages in the life cycle of orchids (outer circle), and the most important ways in which nutrient responses come into being during the various stages of the life cycle. These are: (i) ecophysiological characteristics of the orchid species themselves, varying during the various stages of the life cycle; (ii) growth responses of mycorrhizal fungi; (iii) nutrient effects on establishment of a compatible mycorrhizal association, with an impact of (i) and (ii) but the equilibrium being dissimilar to both; and (iv) nutrient effects on performance of competing species of higher plants. The density of the dotted area symbolizes the intensity of interaction with mycorrhizal fungi. The interactions determining orchid survival most in the various stages are indicated in the inner part of the circle. These are: specificity and recognition phenomena for germinating seeds; mycorrhiza establishment and development for developing seedlings; and competition with surrounding plant species in the adult phase.

Figure 1 presents an overview of the most important factors affecting orchid nutrient responses at the various stages in the life cycle. Nutrient availability may affect orchid growth in various ways: (i) by directly affecting orchid growth, the responses being determined by ecophysiological characteristics of the orchid species, (ii) by affecting growth of associated orchid mycorrhizal fungi, determined by ecophysiological characteristics of the mycorrhizal fungi, (iii) by determining the interaction between mycorrhizal fungi and developing orchids, determined by differences between the responses of both organisms, and (iv) by affecting the light regime via changes in canopy structure as a result of changed competitive abilities among plant species. The four mechanisms are of different importance during the various phases of the life cycle, which is the reason to treat nutrient effects, and especially nutrient metabolism of orchids, in the context of the timing of the life cycle of the separate individuals.

ORCHID LIFE CYCLE (FIGS 2–4)

Orchid fruits contain a vast amount of seeds. These usually are less than 1 mm each, containing a rather undifferentiated embryo about one-tenth of a millimeter in size and only very limited amounts of seed reserves, in the form of lipids, proteins or glycoproteins (Arditti 1967; Manning & Van Staden 1987; Rasmussen 1990).

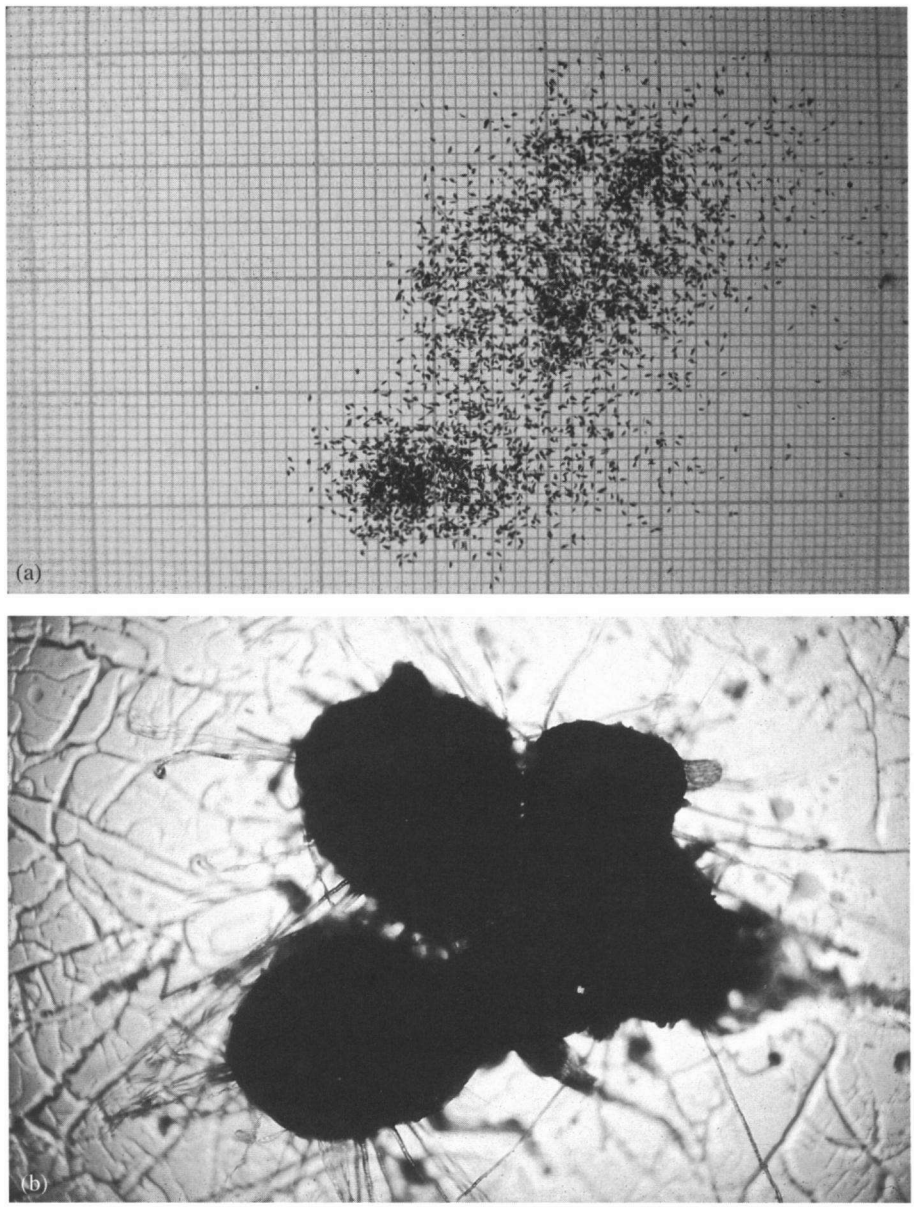

Fig. 2. (a) Seeds of *Dactylorhiza praetermissa* on a background of 1 mm grid. (b) Four-month-old *D. praetermissa* protocorms, grown symbiotically on an agar medium, and photographed against the light. The shoot initial can be distinguished at the top. A hyphal network of the mycorrhizal fungus *Epulorhiza repens* can be distinguished clearly; in- and outcoming hyphae can be observed at the ends of the rhizoids at the protocorm surface. In the right upper protocorm the remains of the seedcoat can be distinguished. Actual size of the protocorms is about 5 mm.

It is generally assumed that the tiny orchid seeds germinate soon after they make contact with the soil and will continue to develop only when washed down sufficiently. Indirect evidence is formed by the fact that germination of European terrestrial orchid species only takes place in darkness (see, e.g. Arditti 1982; Van Waes & Debergh 1986),

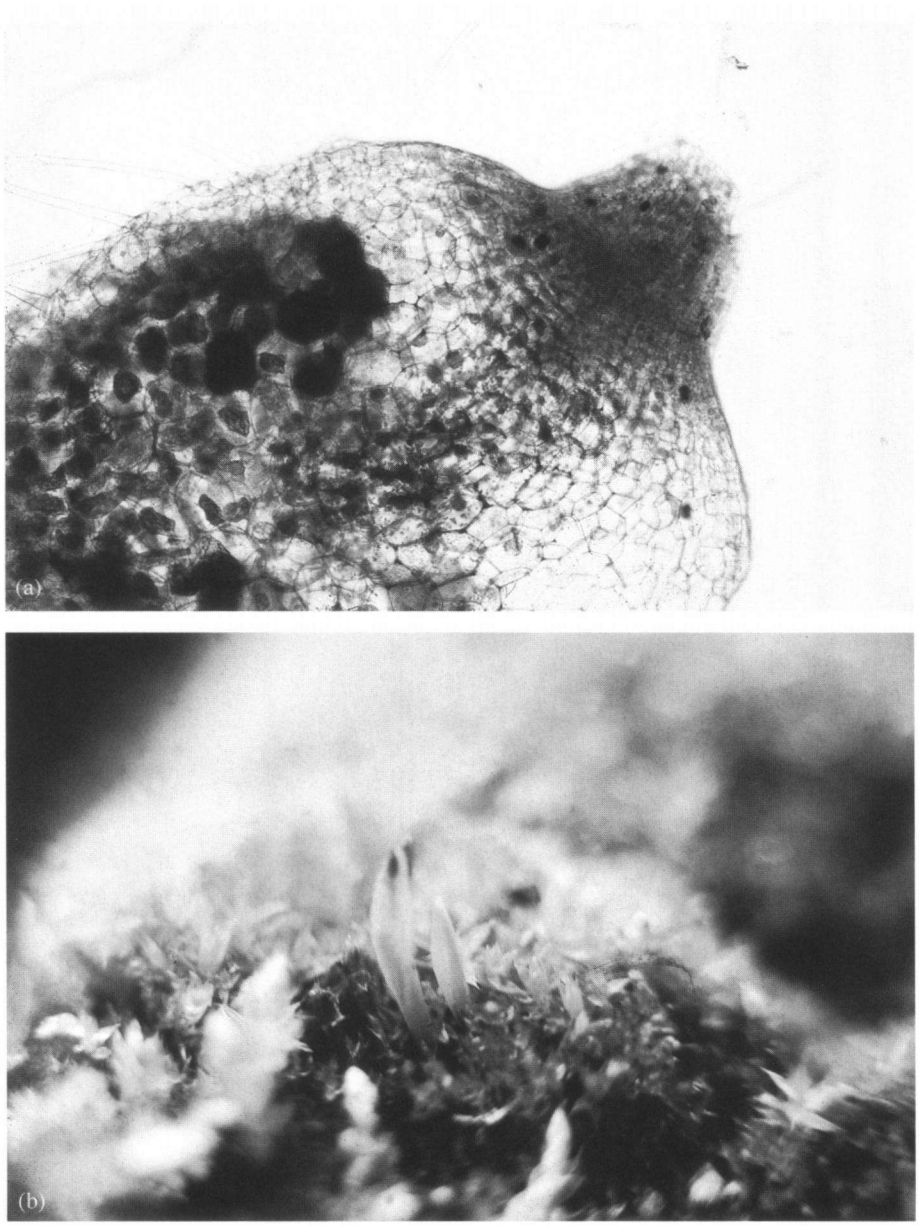

Fig. 3. (a) Cross-section through protocorms of *D. praetermissa*, with upper right the shoot initial and directly below the meristematic region of small cells. In the left lower corner, digested pelotons appear as dark spots. (b) First season *D. praetermissa* seedlings appearing above ground in moss layer in almost natural circumstances.

but is stimulated by a short illumination in advance (Rasmussen *et al.* 1990b); there is, however, only little more direct proof (e.g. Leeson *et al.* 1991 for *Dactylorhiza fuchsii*). It is difficult to assess to what extent orchids are capable of forming a seedbank, because in the usual trials for seedbank composition the presence of compatible orchid mycorrhizal fungi is uncertain, and the time span of such experiments is usually too short to enable seedlings of orchids to appear.

Fig. 4. Flowering individual of *Dactylorhiza praetermissa* in floating rich-fen. (Photographs: E. Dijk).

A common characteristic of orchids lies in early seedling development. After germination the swelling embryo develops into a small conical body, the *protocorm*, which lacks genuine roots and therefore efficient means for independent nutrient uptake, and moreover physiologically shows severe reductions in especially carbon- and nitrogen metabolism. Protocorms of most European terrestrial orchids are completely devoid of chlorophyll which makes them, in combination with the almost absence of seed reserves, completely C-heterotrophic. Like other, tropical, representatives of the *Epidendroidae*, protocorms of *Liparis loeselii*, however, possess chlorophyll in this phase (E. Dijk, personal observation), and theoretically could be capable of a very limited independent development. The reductions in metabolism in this phase of development are overcome by carbon and mineral nutrient translocation by mycorrhizal fungi (see below); this encompasses that mycorrhizal infection is a prerequisite for orchid development in natural circumstances.

From about 2 weeks after germination, the protocorm soon develops rhizoids at its surface, except at the top. At the top, a meristematic ring develops within the protocorm, while later, in the centre below, a longitudinal vascular chord is formed within a few weeks (Harvais & Hadley 1967b; Rasmussen *et al.* 1990a). These are not

infected by the mycorrhizal fungus. Germination of seed of European terrestrials probably takes place in the very top layer of the soil. Studying a population of *Dactylorhiza fuchsii*, Leeson *et al.* (1991) found protocorms most often in grass tufts and moss. During the course of a season the maximum number of protocorms of the smallest size class was present in November, while these were nearly absent in samples taken from April to September. This means that germination takes place less than 3 months after seedshed. This is supported by indirect evidence from germination trials. The optimum temperature observed for germination of *Dactylorhiza majalis* in artificial culture shows a high optimum in the range 20–27°C, dropping to nearly zero at 15°C (Van Waes & Debergh 1986; Rasmussen *et al.* 1990a). This suggests that germination must occur during or before autumn. For further development the optimum temperatures lie somewhat lower, between 20°C and 23°C (see Van Waes & Deberg 1986; Rasmussen *et al.* 1990a; W. C. Evertse, personal communication).

While enlarging, protocorms form a shoot initial at the top of the protocorm in the centre of the meristematic region, which also gives rise to adventitious roots at its base. After some time, in orchids of the subfamily *Orchidoidae*, a first sessile genuine tuber is formed from one of the edges of the meristematic region which, in *Dactylorhiza* species and their relatives, is beet-shaped and ends in a single primary root, in contrast to the tuber of older plants. From this point onwards, the seedlings enter the ever-recurring annual growth cycle that is also characteristic of the adult individuals.

Dactylorhiza species have a strict annual periodicity typical of the temperate climate zone: tubers stay dormant after their formation, which is completed in the middle of the season preceding the one in which the individual will emerge above ground. At this time, the current year's shoot and the tuber from which it has originated (or the protocorm and its small shoot when the annual cycle is entered for the first time) wither away. In culture, 2 or 3 months at *c.* 5°C are adequate for breaking dormancy of the newly formed tuber (cf. Evertse & Stein 1987; Hadley & Pegg 1989), triggering the unfolding of shoots, growth of adventitious roots and, in larger plants, the normal development of flower primordia (Dijk, unpublished data). Formation of a new tuber from an initial begins at the onset of growth after the winter dormancy. Each separate tuber therefore has a life span of more than one season.

Orchis and *Ophrys* species have ovoid tubers both as seedlings and as adult plants that are formed on a sinker. The majority of these round-tubered genera (including *Orchis morio* that has a relatively northern distribution) follow a more Mediterranean growth rhythm. These emerge in autumn or winter and, as far as is known at present, have no need for a chilling period, but are stimulated by lowering of the temperature during autumn (Evertse & Stein 1987). Vegetative reproduction generally plays only a minor role in the tuber-forming genera; in species such as *Herminium monorchis* and *Serapias lingua*, however, a second and normally dormant tuber initial may develop regularly, ultimately giving rise to groups of similar individuals.

In several tuberous species such as *Orchis morio*, *O. mascula* and *Platanthera bifolia* reallocation of reserves from the old to the new tuber have been demonstrated at the end of the growing season (Franz & Meier 1971; see also Ernst & Rodriguez 1984), enabling a gradual build-up of reserves and forming a bottle-neck determining plant performance in the next growing season. In *Orchis simia* (Willems 1982) and *Dactylorhiza majalis* (Dijk & Olff 1994) flowering after appearing above ground appeared to be size-dependent. In *Dactylorhiza* species chance of survival proved to be positively related to plant biomass in hay meadows (Dijk & Grootjans 1998). Dormant tubers are not

infected by mycorrhizal fungi and roots in the following season, therefore, have to be externally reinfected. Taking the great and different impact that mycorrhizal fungi have on orchid nutrient response (see below), also for this reason tuber formation and dormancy period can be considered the major bottle-neck in the annual cycle of tuberous orchids.

Other orchid species, such as *Epipactis helleborine* (Light & MacConaill 1991) and *Cypripedium calceolus* (Kull & Kull 1991), form a short rhizome after the protocorm stage, that annually extends gradually from a perennating bud formed at least a year before emergence of the corresponding shoot. At regular intervals, these species show side-branching of the rhizome giving rise to complete clones. The growing of these clones, in combination with dying back of the rear end thereof, enables a limited means of shifting (maximally some 2 cm/year) of the clone in deteriorating habitat conditions. This has been described for *Epipactis palustris* in dune slacks under diminishing seepage intensity of calcareous groundwater (Grootjans *et al.* 1995). Most rhizomatous species display the temperate growth rhythm described for the round-tubered orchids; *Goodyera repens* is unusual and evergreen.

MYCORRHIZAL ASSOCIATIONS*

Development of mycorrhizal infection

Mycorrhizal infection is restricted to subterranean tissues only, i.e. to the subepidermal zone of protocorm and root parenchyma. Infection of seeds by mycorrhizal fungi takes place during or shortly after germination, in some cases at the basal end of the protocorm via the suspensor cells of the embryo (Clements 1988) or via rhizoids of the protocorm (Hadley 1982; Rasmussen *et al.* 1990a). Below the epidermis a cortex layer of cells is formed, which is rapidly colonized by hyphae of mycorrhizal fungi. In normally functioning mycorrhizas, the hyphal coils branch only sparingly; dense cushion-like branching of hyphal coils is the first symptom of a transition to pathogenic infection by the same mycorrhizal fungi (Beyrle *et al.* 1991). In part of these cells digestion of hyphal coils ('pelotons') occurs, while in others ('host cells') fungal pelotons remain intact (Harvais & Hadley 1967b; Rasmussen *et al.* 1990a). The vascular cord and meristematic region of the protocorm are not infected in normally functioning mycorrhizas. The infection pattern in older plants is strikingly reminiscent of that of protocorms, although the appearance of the mycorrhizal structures is very different. Roots of older European terrestrials are usually heavily infected in the subepidermal parenchyma; as in protocorms the central stele stays fungus-free. Via the rhizoids of roots or protocorms, the plant is connected to the external mycelium. Genuine tubers such as those of the *Orchis*-relatives, sinkers and rhizomes are similarly not infected; the latter means that mycorrhizal infection is discontinuous, and root infections from newly formed tubers are established from new external infections (Hadley 1982). The swollen, tuber-like roots of *Spiranthes*, however, may show mycorrhizal infection in the outer zone (Summerhayes 1951).

After initial infection the development of mycorrhizas can easily derail, and in symbiotic cultures a range of interactions can be met from a loss of mycorrhizal infection via normal symbiotic infections to pathogenic infections (Hadley 1970). In

*Nomenclature and taxonomy of orchid mycorrhizal fungi according to Moore (1987).

both extremes growth of protocorms is stopped, in the first case by the cessation of nutrient flow towards the developing seedling, in the latter by too-vigorous infections followed by parasitism and degradation of orchid tissue by the fungi. While in normally developing symbioses infection is characterized by sparse branching from hyphal coils to neighbouring cells, parasitic infections can be recognized by massive and irregular spreading of hyphae through orchid tissue (cf. Beyrle *et al.* 1991). Both infection types may even occur simultaneously in orchid tissue (Alconero 1969). Besides the collapsing and degradation of hyphal coils in root tissue, the presence of several phytoalexins (fungistatic phenolic compounds) such as orchinol and hircinol tubers of adult Orchidoidae may play a role in preventing infection and depletion of reserves in these storage organs (Fisch *et al.* 1973). Some of the more vigorous isolates (*Ceratorhiza* and *Thanatephorus*), however, have been shown to grow unaffected in concentrations of orchinol well in excess of those found in orchid tubers (Hadley & Pegg 1989). The balance, due to synthesis of polyphenolics by orchids and polyphenoloxidases by mycorrhizal fungi as demonstrated in *Ophrys lutea* roots, seems important in preventing both pathogenic parasitism by the fungus and its elimination from root tissue at the other hand (Pais & Barroso 1983). Exudation of polyphenolics by protocorms under suboptimal culture conditions (see, e.g. Van Waes 1984) may be related to maintenance of such an equilibrium; the exact nature of these compounds and their fungistatic nature needs confirmation, however.

Orchid mycorrhizal fungi

The most frequently observed endophytes in European terrestrial orchids belong to the imperfect genera *Ceratorhiza*, *Moniliopsis* and *Epulorhiza*, in older literature collectively referred to as members of the ill-defined imperfect genus *Rhizoctonia*. Corresponding teleomorphs* of these genera are *Tulasnella*, *Ceratobasidium* and *Thanatephorus*, respectively (cf. Moore 1987). Ecologically, two major types can be distinguished within orchid mycorrhizal fungi. *Ceratorhiza* and *Moniliopsis* are fast-growing biotrophes or necrotrophes, able to use a large range of nitrogen sources including nitrate, ammonium and amino-nitrogen. *Moniliopsis solani* (Kühn) Moore is both an orchid symbiont and a pathogen, causing damping-off diseases on a variety of crop species. Strains isolated as pathogens from culture crops have been shown to be effective symbionts of *Dactylorhiza majalis* subsp. *purpurella* (Harvais & Hadley 1967b). *Moniliopsis* and more so *Ceratorhiza* spp. are common endophytes of various terrestrial orchid species (cf. Warcup & Talbot 1967, 1971, 1980; Hadley 1970; Filipello Marchisio *et al.* 1985; Currah *et al.* 1990); these are among the most aggressive pathogens causing black rot disease (Saksena & Vaartaja 1961; Ypema *et al.* 1987). In artificial *in-vitro* culture they have been shown to be symbiotic with several *Dactylorhiza* species (Harvais & Hadley 1967b; Dijk & Eck 1995c). *Ceratobasidium cornigerum* (named *Ceratorhiza goodyerae-repentis* in its non-perfect, anamorph state) is at least the natural symbiont of *Goodyera repens*: it is almost exclusively isolated from this orchid in natural circumstances (Purves & Hadley 1976; Hadley 1982; Alexander & Hadley 1983, 1984). In *in-vitro* culture, mycorrhizas have been synthesized between this fungus and *Orchis morio* and *O. laxiflora* (Muir 1989).

*Since generative phases of these fungi are seldom met, nomenclature of these species is mostly based on the imperfect stages. Species limitations of the imperfect and perfect stages may conflict; therefore a double nomenclature exists in which the teleomorph name is used only when the corresponding perfect stage is known with certainty.

A second group consists of slower-growing, less pathogenic fungi such as *Epulorhiza* (cf. Saksena & Vaartaja 1961) and *Sebacina* species (Warcup 1981; Milligan & Williams 1988). In particular, *Epulorhiza repens* has been isolated from a vast amount of terrestrial orchids, and is considered an ubiquitous orchid endophyte (Harvais & Hadley 1967a; Filipello Marchisio *et al.* 1985). Strains of this fungus have been found to be unable to use nitrate and/or ammonium as a nitrogen source (Stephen & Fung 1971; Hadley & Ong 1978), which has been considered an adaptation to the root infecting habit (Hadley & Ong 1978; Hadley 1982).

Less regularly, outside the usual *Rhizoctonia*-like fungi, dark sterile mycelia ('MRA') have been isolated from a wide range of terrestrial orchid species including *Platanthera* spp. and *Coeloglossum viride*, perfect stages of which were found in the hyphomycete genera *Phiocephala* and *Leptodontidium* (Currah *et al.* 1990). These mycelia seemed especially common endophytes of *Epipactis helleborine* (Salmia 1989), but whether they can be regarded as genuine mycorrhizal fungi for these orchid species awaits further confirmation by symbiosis tests. Obvious basidiomycetes showing mycelial clamp connections, that have been isolated from various achlorophyllous orchid species (see Harley & Smith 1983), have only seldom been encountered in roots of green terrestrials (but see Currah *et al.* 1990).

Orchid mycorrhizal fungi are not dependent on orchids for their maintenance and are capable of utilizing complex polymers, such as cellulose, as a carbon source (Garrett 1962; Smith 1966; Hadley 1969), enabling an independent existence as soil saprotrophes. Specificity of mycorrhizal symbiosis in European terrestrial orchids has been judged differently. No specificity pattern emerges from isolation trials (Harvais & Hadley 1967a), meaning that root infections are likely to be coincidental or at least that orchids discriminate poorly between fungal strains present in their roots. Only *Goodyera repens* is, in natural circumstances, almost invariably infected with *Ceratorhiza goodyerae-repentis* (Hadley 1970; Purves & Hadley 1976; Alexander & Hadley 1984). On the other hand this species, like many others, can be forced to accept various types of endophyte, which has been the main argument for claiming non-specificity of mycorrhizal infection (Hadley 1970). For Australian terrestrials (Warcup 1971) and later also for European ones (Clements *et al.* 1986; Muir 1989) some degree of specificity has been claimed, the various fungal strains being, to a different extent, beneficial for distinct orchid groups. Since *Ceratorhiza*-like fungi (found symbiotic with *Dactylorhiza* and *Orchis morio*) have very different effects on orchid nutrient responses (see below) than *Epulorhiza*-like strains (different strains being most beneficial for distinct groups such as *Ophrys* and several *Orchis* and *Serapias* species), the unsolved specificity problem is of direct relevance for studying the ecology of these species. Specificity in mycorrhizal association between groups of orchids and fungi may be underestimated in *in-vitro* symbiosis trials. *In-vitro Spiranthes sinensis* may be mycorrhizal with very different types of mycorrhizal strains (Masuhara *et al.* 1993); in an elegantly designed experiment adult plants and protocorms in the soil were shown to be almost exclusively infected with *Epulorhiza repens*, while the other strain types tested were shown to be present (Masuhara & Katsuya 1994).

Although it has often been suggested, there exists no direct reason to relate the rarity of many orchid species to strict habitat requirements of the mycorrhizal fungi which, in turn, for reason of the obligate character of the symbiosis, would lead indirectly to equally strict requirements of the associated orchid species. The vast number of ecologically distinct orchid species from which the more common endophytes have been

isolated (e.g. Harvais & Hadley 1967a; Hadley 1970; Hadley & Williamson 1972; Filipello Marchisio *et al.* 1985) suggests that they tolerate a much broader range of edaphic circumstances than the orchid species with whom they were found associated could ever be capable of; if this was the key factor for orchid distribution a similar distribution of orchid species and corresponding mycorrhizal fungi should be expected. Many of the fungi are regularly encountered in all kinds of environments, including in culture (Ypema *et al.* 1987) and forest nurseries (Saksena & Vaartaja 1961) in the absence of orchid species. Strains isolated as pathogens of culture crops have proved successful symbionts of *Dactylorhiza majalis* ssp. *purpurella* (Hadley 1970, 1984). Seeds of *Spiranthes sinensis* did not germinate on all test sites where its natural fungal associate was shown to be present (Masuhara & Katsuya 1994). Although the possibility of strain-specific orchid response to morphologically similar, but symbiotically distinct strains within the same fungus species cannot be ruled out, it appears that in orchid mycorrhizas the fungi are far less exacting than their symbiotic associates (cf. Harley & Smith 1983).

NUTRIENT METABOLISM AND RESPONSES

Germination and seedling phase

The limited reserves of orchid seeds are mobilized only in the presence of free sugars (Manning & Van Staden 1987), for which the infection with symbiotic fungi seems a prerequisite under natural circumstances. Non-mycorrhizal nutrient uptake of orchid seedlings in the heterotrophic protocorm stage must be limited. Because of the small dimensions, protocorms will be able to exploit only a space of some mm in this phase, including that covered by rhizoids. Especially when taking the poor mobility of ammonium and phosphate in the soil into account, the available fraction of these ions will therefore soon be exhausted. The hyphal network formed around the developing protocorm (see Fig. 2b) will extend this range considerably. Even without taking these spatial effects into account, nitrogen uptake of developing protocorms can be expected to be low since several reductions exist in the nitrogen metabolism, which are reflected by nutrient requirements in non-symbiotic culture.

Orchid nitrogen metabolism is very complex, and seems at first instance to be determined largely by the stage of development and therefore by the extent to which individuals rely on parasitism (mycotrophy) within the mycorrhizal association. Most European terrestrial orchids require amino nitrogen in the protocorm stage, which could be met largely by glutamic acid for *Dactylorhiza majalis* ssp. *purpurella* (Harvais & Raitsakas 1975) or glutamine in *Ophrys sphegodes* and *Orchis laxiflora* (Mead & Bulard 1975, 1979) and other European terrestrials (Van Waes & Debergh 1986). Later in the protocorm stage, these amino acids (or complex additives containing these, such as, for example, yeast extract) may have an adverse effect on non-symbiotic *in-vitro* culture, since they appear to elicit the formation of polyphenolics (S. Pais and W. C. Evertse, personal communication), that may play a role in balancing the two partners within the mycorrhizal association (Pais & Barroso 1983). Probably the ability of orchids to use mineral nitrogen sources evolves only during the course of development, and reductions in nitrogen metabolism are probably related to the parasitic nature of the symbiosis in this stage, such as observed in parasites lacking chlorophyll such as *Orobanche* (Press *et al.* 1986). Unfortunately, no data exist on the nature of nitrogenous

compounds translocated from mycorrhizal fungus to orchid seedlings, but it can be assumed to be in the form of glutamine or glutamic acid.

The inhibitory effects of ammonium and especially nitrate nitrogen on germination and protocorm development of *Orchis laxiflora* (Mead & Bulard 1979) and *Dactylorhiza maculata* (Van Waes & Debergh 1986) demonstrates the reductions in N-metabolism in the heterotrophic phase. The fact that in older *O. laxiflora* seedlings ammonium suppletion in the presence of amino acids, in contrast to nitrate, stimulates their development, reflects the order of appearance of nitrate and ammonium metabolizing enzymes (cf. Arditti & Ernst 1984), rather than indicating an ecophysiological adaptation with respect to preference for the dominant nitrogen form in its natural habitat of calcareous seepage swamps. The same explanation might be true for the fact that addition of nitrate nitrogen, more than that of ammonium, decreases germination percentages of *D. maculata* (Van Waes 1984). The tropical epiphyte *Cattleya* was shown to be unable to use nitrate as a nitrogen source during the first 60 days of development, while after that the suitability of this nitrogen source was reflected in the appearance of nitrate reductase (Raghavan & Torrey 1964). In contrast to adult plants, small protocorms of three *Dactylorhiza* species did not show a detectable activity of mineral nitrogen metabolizing enzymes (Dijk & Eck, unpublished data). Preferential uptake of ammonium compared to nitrate in the first phase of development in *Dendrobium* tissues probably has a similar background (Hew *et al.* 1988).

Orchids are clearly highly specialized ecologically to limited ranges of edaphic circumstances. As a result, ecophysiological adaptations to the prevailing edaphic circumstances can be expected. Indeed, pronounced differences in response to nutrient concentration in non-symbiotic growth have been demonstrated between several orchid species from moist hayfields (Fig. 5; see also Dijk & Eck 1995a, 1995c). *Orchis morio* and *Dactylorhiza praetermissa* show a relatively high N-optimum, accounting for their success in fertile habitats such as fertile meadows and hayfields. The restraint of *D. maculata* to low productive heaths, fen meadows and small sedge swamps corresponds to a low N-optimum.

Preferences for nitrogen species might help to explain the limitation of separate species to narrow ranges in soil pH, and between-species differences in optima of soil acidity. In acid soils, especially when waterlogged, decrease in pH will shift the ratio of ammonium and nitrate nitrogen in favour of the former, since both low pH and pE impair ammonification to a much lesser degree than nitrification (cf. Haynes & Goh 1978). Calcifuge plant species have been shown to be inhibited by high concentrations of nitrate, while high ammonium levels may decrease the growth of calcicole plant species (Gigon & Rorison 1972). In contrast to nitrate, ammonium ions were shown to be toxic to seedlings of the calcicole *Dactylorhiza incarnata* during the first year of development, the more so the lower the pH. Contrastingly, nitrate had a positive or at least did not have an adverse effect at the same range of concentrations (Dijk & Eck 1995b; Fig. 6a). The fact that ammonium toxicity is decreased by high pH is probably responsible for the fact that the species is confined to base-rich environments, since NH_4^+-ions can be present in substantial amounts in inland calcareous swamps harbouring this species (Dijk & Grootjans 1998). In contrast to medium pH, calcium levels as such did not have an effect on the toxicity of ammonium ions. This mechanism is probably also responsible for confining the species to early stages of succession in calcareous wet dune slacks, since during the course of succession organic matter content, and hence N-availability, increase while soil pH decreases; the result of this will

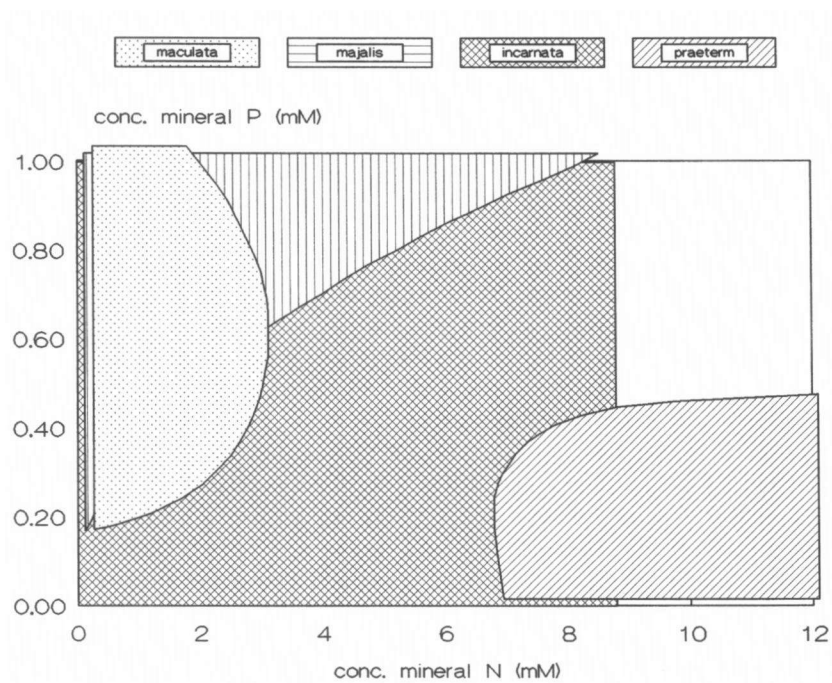

Fig. 5. Differentiation in N- (*X*-axis) and P- (*Y*-axis) optima of seedlings of four *Dactylorhiza* species in their first year of asymbiotic growth. Areas have been indicated where growth of *D. majalis* (densely hatched), *D. incarnata* (checked hatching), *D. praetermissa* (obliquely hatched) and *D. maculata* (dotted) exceeds 75% of the species maximum. After Dijk & Eck (1995a).

be that ammonium levels will be considerably higher in these environments at the later stages of vegetation succession (Olff *et al.* 1993; Grootjans *et al.* 1995; Dijk & Eck 1995b). NH_4^+-ions are also toxic to older seedlings of the far less calcicole *D. maculata* ssp. *maculata*, albeit to a considerably less degree than in *D. incarnata* (E. Dijk, W. Van Eeken & W. C. Evertse, unpublished data; Fig. 6b). Moderate nitrate dosages in the same range of concentrations had a positive effect on growth. Also for this species ammonium toxicity forms a good explanation for the observed distribution patterns in the field. In heaths and heath-related grasslands the species is limited to places with high pH of the groundwater and high base saturation of the soil, correlated to a low ratio between ammonium and nitrate N (Roelofs *et al.* 1996); like other character species from matgrass swards on soils with relatively high base saturation, adult *D. maculata* plants are sensitive to increases in ammonium availability (Houdijk 1990). In brook-valley hay meadows, the species is limited to low-productive small sedge vegetation, associated with low total N content of the soil and a not-too-high availability of ammonium (Dijk & Grootjans 1998).

Effects of mycorrhizal infection on nutrient responses in the seedling phase

The primary function of mycorrhizal infection in the youth phase lies in the transport of C-compounds to the developing seedlings; in the absence of an easy accessible carbon source, growth of the C-heterotrophic protocorms only commenced after mycorrhizal infection. Translocation of sugars towards protocorms has been demonstrated by radioactive labelling in classical studies on *Dactylorhiza* protocorms symbiotic with *Epulorhiza repens* (Smith 1966) and *Goodyera repens* symbiotic with *Ceratobasidium*

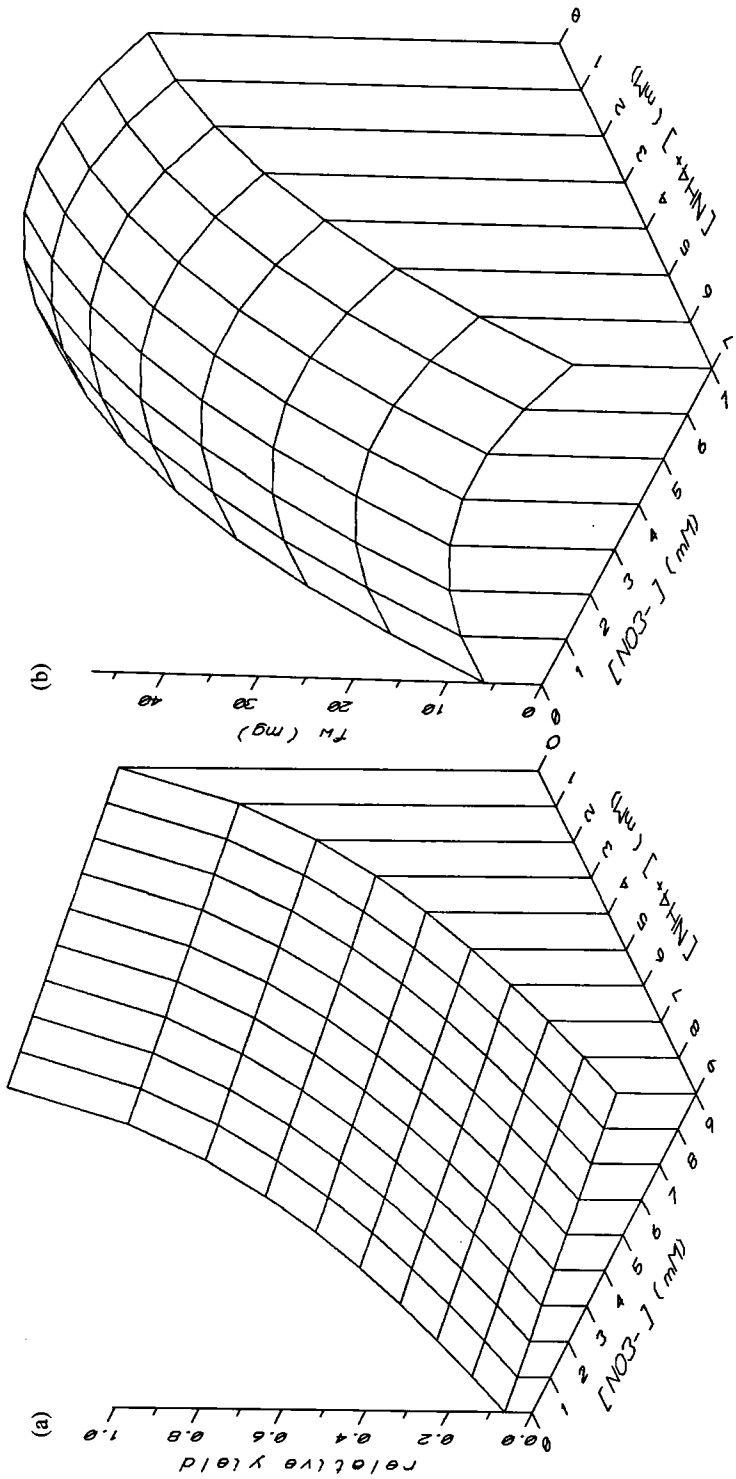

Fig. 6. Ammonium and nitrate response of (a) *Dactylorhiza incarnata* protocorms from a calcareous dune slack (Noordvaarder, Terschelling; after Dijk & Eck 1995b) and of (b) *Dactylorhiza maculata* ssp. *maculata* seedlings from a moist matgrass sward (Westerholt, Drenthe; after Dijk, Van Eeken & Evertse, unpublished). (a) gives relative fresh weights of surviving protocorms per test-tube; these were inoculated with five protocorms each after a culture period of 22 weeks starting 2 months after germination. *Y*-axis in (b) shows fresh weight of separate individuals, averaged over *c.* 50 seedlings per culture vessel after 1·5 years of culture, starting 6 months after germination.

cornigerum (Purves & Hadley 1975; Alexander & Hadley 1985). Evidence suggests that sugars are translocated as trehalose, which is transformed to sucrose in the seedling (Smith 1967; Hadley 1984), thus forming a continuous source-sink system. The translocated sugars may originate from decomposition of complex polymers such as cellulose, which both in soil (Garrett 1962) and in artificial cultures (Hadley 1969) can be used as a carbon source by the mycorrhizal fungi. In normally functioning mycorrhizas radioactive labelling from $^{14}CO_2$ supplied to green shoots of *G. repens* did not appear in the mycelium of its mycorrhizal fungus *C. cornigerum*, and normally therefore no transport of sugars seems to take place from plant to fungus (Hadley & Purves 1974; see also Harley & Smith 1983) as in other types of mycorrhiza, demonstrating the parasitic nature of orchid mycorrhizas. ^{14}C from dead seedlings, however, soon appeared in the hyphae of the fungus, probably as a result of necrotrophe degradation by the same mycorrhizal fungus.

The role of carbohydrate is not only that of translocated nutrient between orchid and mycorrhizal associate. Although orchids may easily tolerate high levels of sugars (up to 20 mg l^{-1}) in the culture media (Van Waes 1984), mycorrhizal cultures only develop well when carbon availability is limited: higher levels of easy accessible carbon sources have been shown to stimulate fungus growth and derail mycorrhizal development by enhancing pathogenic infections (Harvais & Hadley 1967b; Hadley 1969; Williamson & Hadley 1970; Hadley & Williamson 1971; Purves & Hadley 1976).

Apart from interfering with the carbon metabolism, mycorrhizal infection has a pronounced influence on the uptake of mineral macronutrients. Smith (1966) demonstrated phosphate transport from *Epulorhiza repens* to *Dactylorhiza majalis* ssp. *purpurella* protocorms using radioactively labelled ^{35}P. In *Goodyera repens*, seedlings mycorrhizal with *Ceratobasidium cornigerum* had higher N and P contents, and higher growth rates than non-mycorrhizal or fungicide treated ones (Alexander & Hadley 1984). Both *Epulorhiza repens* and/or *Ceratorhiza* fungi stimulated growth of seedlings of *Dactylorhiza* species and *Orchis morio* at low nitrogen (NH_4NO_3) availability, in comparison to seedlings in non-symbiotic culture (Fig. 7, Dijk & Eck 1995c).

Nutrient availability is not only of interest in a physiological context, but appears to be at least as important ecologically in regulating the interaction between developing seedlings and mycorrhizal fungi. This interaction may range from pronounced growth stimulation to induced mortality of seedlings (Hadley 1970). A high nutrient status (Gilligan & Simons 1987) and more specifically nitrogen availability (Weinhold *et al.* 1969, 1972) has been shown to increase infectivity and virulence of *Moniliopsis solani* in pathogenicity trials. In *Dactylorhiza incarnata* seedlings, symbiotic with a *Ceratorhiza*-like isolate, increased mortality was demonstrated at high nitrogen levels, independent of whether the nitrogen was given as nitrate, ammonium or amino nitrogen (Beyrle *et al.* 1991). There is no sign that deteriorated growth of either of the mycorrhizal partners is responsible for the disrupted functioning of orchid mycorrhizas at a higher nutrient availability. When grown asymbiotically, orchids may tolerate or even benefit from nutrient levels that are lethal when grown symbiotically. This means that the sensitivity for high nutrient levels in symbiotic culture originates from the effect that N-availability to the fungus has on the interaction between partners of the symbiosis (cf. Dijk 1989; Beyrle *et al.* 1991; Dijk & Eck 1995c). The outcome of the interaction is not only dependent on external nutrient levels, but also on the type of mycorrhizal fungus that forms part of the symbiosis. While growth of *Dactylorhiza* seedlings was stimulated

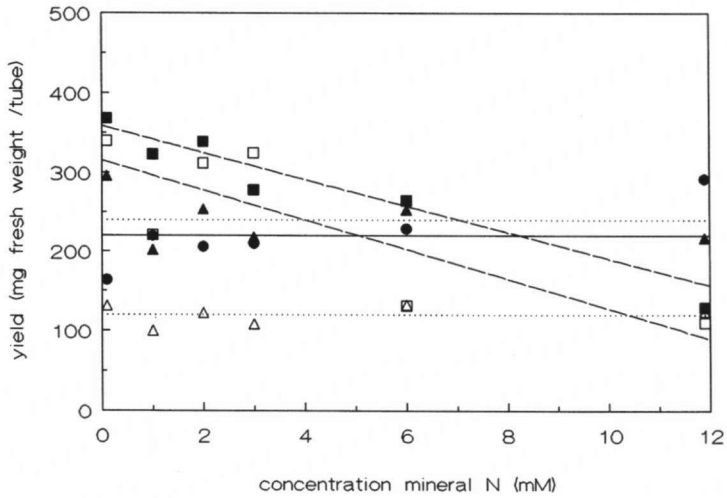

Fig. 7. Effect of mycorrhizal infection on nitrogen response of *Dactylorhiza majalis*. Responses are given in non-symbiotic culture (dot markers and unbroken lines), and in symbiotic culture with two different strains of *Epulorhiza repens* (triangular markers and stippled lines) and two of *Ceratorhiza* spec. (square markers and broken lines). Although growth rates differ, the nitrogen response in *Epulorhiza* symbioses is similar to that in non-symbiotic culture. In *Ceratorhiza* symbioses growth is stimulated at high, but impeded at higher N concentrations. Cultures mycorrhizal with these strains show a significantly more negative N response. After Dijk & Eck (1995c).

at low N-concentrations, the fast-growing and relatively pathogenic *Ceratorhiza* fungi had a reverse effect at higher N levels by increasing mortality and decreasing yield of the seedlings. In symbioses with the less pathogenic *Epulorhiza* fungi, the advantage of symbiotic seedlings only diminished in comparison to non-symbiotic ones (Dijk & Eck 1995c).

Nutrient responses and metabolism in adult plants

As adult plants, in several orchid species high nutrient availability has been shown to be detrimental for plant performance. In *Juncus acutiflorus* hay meadows in which N, P, and K simultaneously limited overall standing crop, performance of *Dactylorhiza majalis* was affected negatively as a result of N (NH_4NO_3) and P, but not K application. Decrease in orchid frequency could not be explained solely by increase in biomass of its main competitors, suggesting that other mechanisms are responsible for its decline in numbers (Dijk & Olff 1994). Fertilization with nitrate N and inorganic P and K in chalk grasslands led to disappearance of all orchid species within 3–4 years after its application; a decade after cessation of the fertilization treatment invading *Dactylorhiza fuchsii* individuals appeared in all plots including those fertilized with NO_3^-, but not in plots that received high phosphate levels (J. H. Willems, personal observation). Fertilization with N and P in pot cultures, i.e. without competition of other plant species, reduced survival of *D. fuchsii* plants in their first year above ground (McKendrick 1994, 1996), implying that for this species again competition is not the only mechanism by which growth is impeded. In *Orchis morio*, however, a plant from fertile hay meadows, no negative effect on growth could be detected in this same experimental set-up and at the same fertilizer concentrations. At much higher N and P dosages, decreases in frequency of flowering individuals of this species was observed in

the field (Silvertown *et al.* 1994). Although flowering incidence was associated with a strong increase in the standing crop of the surrounding vegetation, the orchid response to high P levels in particular seemed disproportionally strong in comparison to that of the surrounding sward.

Growth rates of older *Goodyera repens* seedlings and the N- and P-contents of infected plants were higher in untreated plants than in plants whose external mycelium was destroyed by fungicide (Alexander & Hadley 1984), indicating a perpetuated role of the mycorrhizal fungi in nutrient uptake in the adult phase. P transport over the mycelium was demonstrated over distances of 9 cm in this symbiosis, and P-uptake was up to 100 times greater in infected plants than in uninfected ones, probably as a result of better substrate utilization by the mycelial network. As a result plants with an intact mycelium showed higher growth rates at low P availability than fungicide-treated plants, but no differences were observed in P-rich conditions (Alexander *et al.* 1984). Similarly, 2-year-old symbiotic *Dactylorhiza majalis* plantlets grown in pot culture without nutrient stress grew equally well, whether or not the external mycelium was drenched in fungicide (Hadley & Pegg 1989).

Alexander & Hadley (1985) showed that in *Goodyera repens* only protocorms and seedlings obtained labelled carbohydrates from *Ceratobasidium cornigerum*, whereas in older, autotrophic plants the transport of C-compounds ceased. At some point in the development of individuals, therefore, a transition from complete dependence from mycorrhizal fungi to a situation more resembling that of other types of mycorrhiza occurs (cf. Hadley & Pegg 1989). Obviously, in 'saprotrophe' orchids that also in the adult phase contain hardly any chlorophyll, such as *Neottia nidus-avis* and *Corallorhiza trifida*, the C-translocation from fungus to plant cannot be switched off. *Epipactis helleborine*, of which regularly chlorotic (Salmia 1986) and steadily lasting (E. Dijk, personal observation) clones are observed, would serve as an excellent test plant with respect to the regulation of carbon and mineral nutrient transport between host and mycorrhizal fungus.

Long-term demographic monitoring studies have revealed the phenomenon of 'dormancy' of adult plants, meaning that aerial shoots of already established plants may remain absent for one or a few subsequent years (Wells 1967). This has been observed both in short-lived species, such as *Ophrys sphegodes* (Hutchings 1987a, 1989) and *Coeloglossum viride* (Willems *et al.* 1997), and in longer-lived species such as *Spiranthes spiralis* (Wells 1967, 1981), *Orchis simia* (Willems 1982) and *Dactylorhiza incarnata* (Tamm 1972, 1991). Especially in short-lived species, dormant plants have a relatively high risk of mortality (Hutchings 1989); in contrast, two-thirds of *Epipactis helleborine* individuals that had stayed dormant for 3 consecutive years proved to be alive (Light & MacConaill 1991). There exists no reliable physiological or demographic evidence for the suggestion that flowering plants may need a rest period after flowering (e.g. Inghe & Tamme 1988) to resume above-ground development. Since photosynthesis is impossible, the only way to recover reserves would be by means of falling back on mycorrhizal fungi (mycotrophy). This can, however, only be demonstrated by determining biomass and nutrient reserves before and after the dormant period. In old literature increase in polysaccharide contents up to 25% and in nitrogen content up to 50% have been given for *Dactylorhiza* during the dormant winter season, suggesting considerable activity of mycorrhizas in plant nourishment during the underground rest period (Fuchs & Ziegenspeck 1927; Ziegenspeck 1936). In the absence of similar, more recent data, a plausible alternative explanation for the existence of the prolonged dormancy period is

that plants may have missed or did not respond to the stimuli triggering reappearance above ground.

BOTTLE-NECKS WITH RESPECT TO LIFE CYCLE STRATEGIES

With only a few exceptions, orchid species are declining rapidly in The Netherlands as well as in other parts of north-western Europe. Examples are species such as *Coeloglossum viride* and *Dactylorhiza incarnata*, which have declined dramatically in numbers in The Netherlands since 1950 (Mennema *et al.* 1985). From 1980 onwards the decline still continues, the species mentioned having disappeared from most of the heath-related matgrass swards on the predominantly calcareous soils in the south, and especially from inland seepage swamps, respectively (see Fig. 8a,b; data from C.A.J. Kreutz in Dekker *et al.* 1996). Apart from more trivial causes such as habitat destruction, for many species eutrophication by fertilization and 'acid rain' and drainage have been held responsible for the sharp decline in numbers of these (Dijk & Eck 1995b; Grootjans *et al.* 1988; Schwertz *et al.* 1996) and many more orchid species (Mennema *et al.* 1980, 1985). These factors all have important repercussions on availability of, especially, nitrogen and phosphate, be it directly as in the case of fertilization and ammonium deposition, or indirectly as in the case of acidification due to loss of seepage intensity of calcium-rich groundwater or following deposition of ammonium salts. It has become clear from field studies that eutrophication is a main cause of population decline (Dijk & Olff 1994; Silvertown *et al.* 1994).

We have reviewed nutrient responses of orchid species at several stages of their life cycle, suggesting that we pointed at a key factor in their ecology. We now ask the question of where the main bottle-necks are situated in the life cycle, in view of the nutrient responses, in order to identify and assess the importance of bottle-necks in survival with respect to nutrient availability in different groups of species.

Only a few studies have been published on life history and population development in the field during the protocorm stage: demographic studies generally have been focused on the above-ground phase. Generally, a prolonged period of time is needed to pass the protocorm stage, but in the light of improved (symbiotic) culture techniques the time lapse between germination and above-ground appearance might be overestimated. For the relatively long-lived species *Spiranthes spiralis*, Summerhayes (1951), Wells (1981) and J.H. Willems (personal observation) estimated the time between germination and above-ground appearance to be between 10 and 14 years, as derived from population changes following a change in management; it remains unclear, however, at what time in this period successful germination of seeds has actually commenced. Plants that appeared above ground flowered either in the same year or within a few years thereafter. Wells & Kretz (1987), however, mentioned a period of 5 years for asymbiotically grown seeds of *S. spiralis* to flower. For *Orchis simia*, the first seedlings in the field appeared above ground 3 years after a single parent plant had fruited and produced seeds (Willems 1982). The results of Leeson *et al* (1991) suggest that *Dactylorhiza fuchsii* reaches the stage in which a single undivided tuber and the first leaves are present in the second year following germination, and therefore the first season is spent as a protocorm. As a result a larger, bifurcate tuber could only be formed 1 year later. Since flowering only takes place on mature, divided tubers, reaching the adult stage of marsh orchids will take at least 3 years. In a population study of *Dactylorhiza praetermissa* flowering individuals reappeared after extinction by

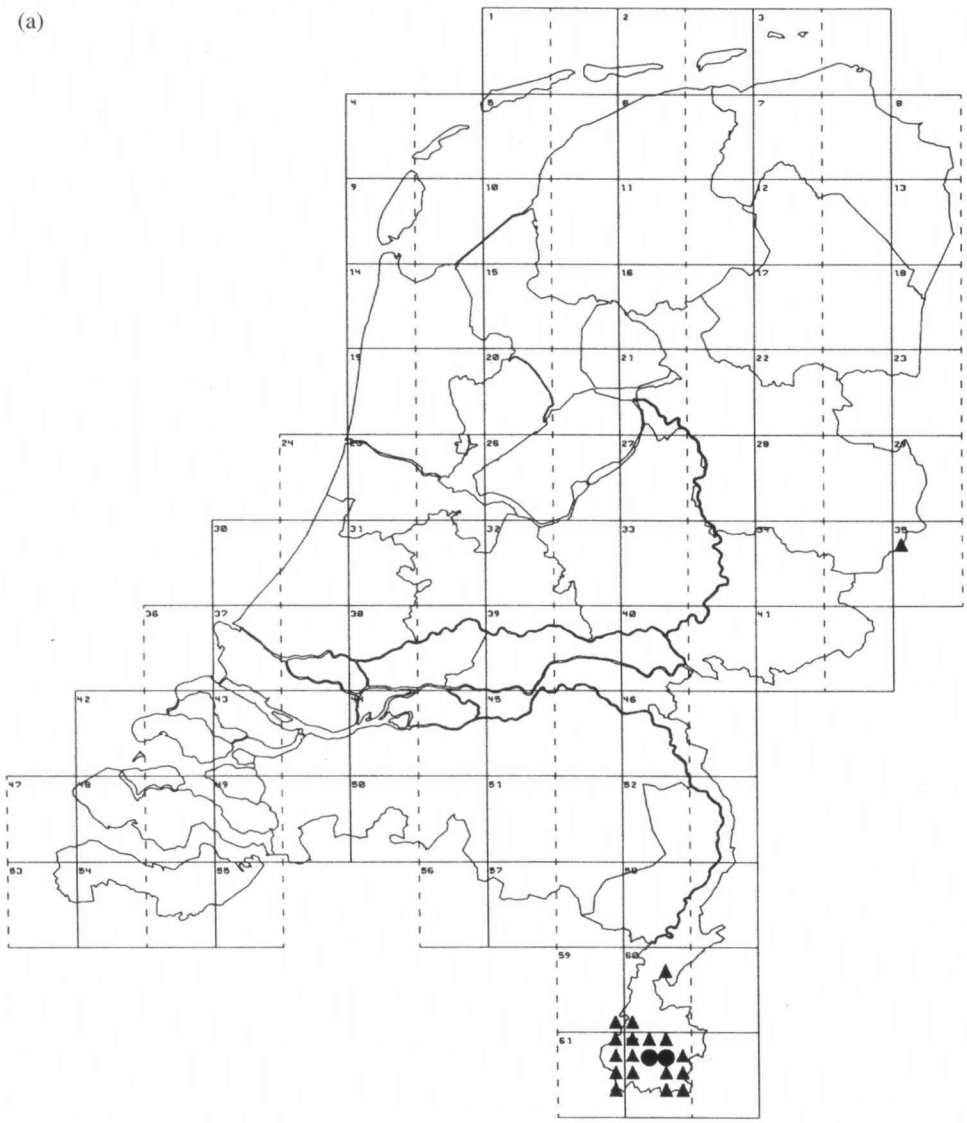

Fig. 8.

inundation after 3–4 years (Vanhecke 1991). This period is in agreement with the time span needed in artificial circumstances for *D. majalis* and *D. praetermissa* (cf. Dijk & Grootjans 1998).

Wells (1981) was the first to publish survivorship (or actually depletion) curves of populations of adult, above-ground plants of several orchid species. For *Spiranthes spiralis* the observations of different populations revealed half-life times varying between 4·6 and 9·2 years, with an average value of about 7 years. This means that it takes, on average, 42 years before the last specimen of a newly established cohort of 50 individuals will have disappeared. Another example of a species with potentially long-lived plants is *Orchis simia* (Willems & Bik 1991), a single individual of which was seen to survive for

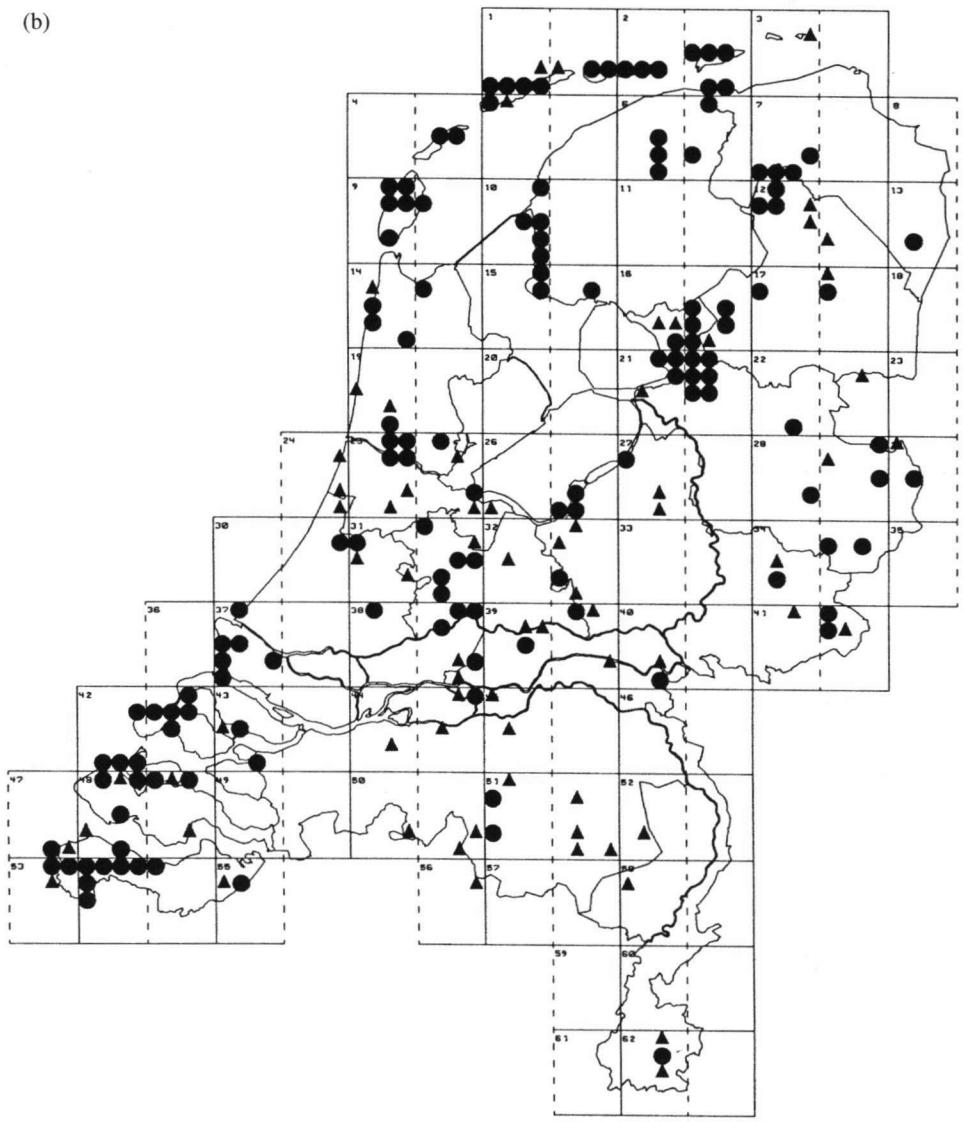

Fig. 8. Distribution patterns of (a) *Coeloglossum viride*, and (b) *Dactylorhiza incarnata* in The Netherlands during the period 1950–80 (triangular markers) and during the period 1980–94 (overlain large dot markers). *C. viride*, a short-living species from heath-related grasslands in calcareous context, has disappeared from most of its known locations in the south of The Netherlands. The potentially long-lived species *D. incarnata* has disappeared from many inland sedge swamps fed by seepage of base-rich groundwater; decline in the coastal area in wet dune slacks on calcareous sand is less pronounced. After Mennema *et al.* (1980, 1985), and Dekker *et al.* (1996), the latter based on data of C.A.J. Kreutz.

at least 25 years in The Netherlands. On the other hand, the analysis of data on seven years of development of populations of *Coeloglossum viride* in The Netherlands revealed average half-life times of only 1·5 years (Willems *et al.* 1997). Similarly, Hutchings (1987a) mentioned a half-life time for populations of *Ophrys sphegodes* of, on average, 1·9 years.

Short-lived species show a high percentage of flowering during the first year of above-ground appearance. According to Hutchings (1987b, 1989), over 70% of *Ophyrs sphegodes* individuals flower in the first year of emergence above ground, and the majority of the flowers in a population are produced by plants in their first 2 years above ground. Forty-three percent of *Coeloglossum viride* individuals that have emerged above ground for the first time flower the very same year (Willems *et al.* 1987). This encompasses that, for these species, in the absence of above-ground organs much of the reserves needed for completing the adult life cycle are gained by mycorrhizal activity. In European terrestrial orchids, short-lived species tend to produce a higher number of seeds than longer-living species. Seed capsules of *Coeloglossum viride*, a species with a mean life span of *c.* 3 years and showing almost no vegetative reproduction, contained on average 2300 seeds (Willems *et al.* 1997). On average 705 and 850 seeds per capsule, respectively, were measured in two successive years for the longer-living *Spiranthes spiralis*, and 860 for *Listera ovata*, an extremely long-lived species. Possibly as a consequence of the phenology of the species, seed production per fruit seemed not to be limited by nutrient resources in *S. spiralis*, but rather by weather conditions and pollinator activity (Willems & Lahtinen 1997, this issue).

Thus, a very important phenomenon to be considered in view of conservation practices is the variability of the lifetime of species within the Orchidaceae. In general, in their reproductive strategy orchids seem to resemble r-selected species in that vast amounts of seeds are formed, reflecting a poor predictability of the environment in terms of presence of suitable mycorrhizal fungi, or probably more important, in edaphic conditions suitable for a normal development of the symbiosis with the particular mycorrhizal strain encountered. Nutrient requirements of mycorrhizal fungi are far less restricted than those of the orchid species with which they are associated (Harley & Smith 1983). Therefore, the role of supra-optimal nutrient concentrations is likely to affect growth and development of orchids more than the growth or even the mere presence of suitable mycorrhizal fungi. This means that, especially in the protocorm phase (in which nutrient requirements are the most pronounced), effects of sub- or supraoptimal nutrient concentrations must be expressed directly in orchid growth (expressed in artificial circumstances by nutrient requirements in non-symbiotic cultures), or indirectly by determining the kind of interaction with symbiotic fungi.

Although for separate combinations between orchids and fungal strains balances are extremely fine-tuned with respect to, especially, C- (Hadley 1970, 1982; Hadley & Pegg 1989) and (ecologically more relevant) N-availability (Beyrle *et al.* 1991; Dijk & Eck 1995c), a just as fine-tuned evolutionary overall adaptation by the orchids is improbable in the light of the large amount of seeds formed by each individual each year (Harley & Smith 1983). Moreover, it is virtually impossible and probably even contra-adaptive to specialize too rigorously, in the light of the existence of poorly predictable strain specific differences in growth rates, instability of symbiotic ability (Alexander & Hadley 1983) and differences between fungi in pathogenicity as a function of nutrient availability (Dijk & Eck 1995c). In the heterotrophic juvenile phase disruption of the balance between the mycorrhizal partners is likely to be more damaging than in the adult phase (McKendrick 1996). The r-strategy, and corresponding high mortality in this phase of the life cycle, therefore, can be considered an optimal strategy to cope with the highly complex and therefore unpredictable interaction between genetic differences between strains in symbiotic ability and pathogenicity, and all environmental

factors affecting these. There are clear limits to the adaptiveness of this strategy, reached at nutrient availabilities which exclude proper mycorrhiza formation, although asymbiotically these would not necessarily be toxic. Although all orchid species have the high risk of juvenile mortality in common, the length of this period may vary considerably from species to species; together with differences in longevity of adult individuals this determines the relative importance of the juvenile mortality in the overall life cycle.

In later stages of the life cycle species differentiate in short-lived and long-lived species. For the relatively short-lived species, stress in the life cycle is put on the repeated obligation to form new mycorrhizas for the establishment of new seedlings in more or less disturbed environments. For this reason, mycorrhiza formation with the less vigorous fungi would diminish risks in the vulnerable, prominent phase in their life cycle. In adult life, however, the advantage in relation to competition to the surrounding sward can be expected to be less, leading to a selective disadvantage in established vegetation in constant circumstances. Many *Ophrys* species, short-lived (Hutchings 1987a,b; 1989), associated with *Epulorhiza* fungi (Clements *et al.* 1986), and having their main distribution in Mediterranean *garigue* and *phrygana* (vegetation formed at forest degradation by irregular burning and heavy grazing), might form a good example of such a strategy.

For the long-lived species, showing several characteristics of a *K*-strategy, another game starts after having passed the juvenile stage. In this phase, the most important interaction is formed by competition (particularly for light) with the surrounding canopy, in which the symbiotic association is advantageous where nutrient availability is limiting and/or competition is more pronounced. At this stage the symbiotic association with the most vigorous fungi would form a selective advantage. Longer-living species can be expected to evolve into the direction of mycorrhizal symbioses with these fungi, as seems to have occurred in *Dactylorhiza* species and in *Goodyera repens*, for which *Ceratorhiza* fungi seem the most efficient mycorrhizal associates (Clements *et al.* 1986; Hadley 1982; Alexander & Hadley 1984, 1985; Dijk & Eck 1995c). For these symbioses, the risk of disruption of the symbiosis in the youth phase is much more pronounced (see Dijk & Eck 1995c), so that to a stronger degree than in the aforementioned example, the protocorm phase is a fine-tuned expression of edaphic circumstances in the field in comparison to the adult phase. This means that population decline of adult individuals is, rather, a final signal of disturbed and deteriorating habitat conditions than an early warning thereof. In particular for the latter species group constant nature management is required, in which nutrient availability is constantly kept at the lower edge of the tolerated range of adult individuals, in order to express the selective advantage of mycorrhizal infection in adult individuals and to minimize the risk for developing ones.

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